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14. ABSTRACT

An intriguing discovery reported in Science Magazine, in 2004 by Crookes and colleagues, outlined the first look at an unusual family of natural protein-based polymers called reflectins. Reflectins are characterized by unique spectral and optical properties. Our interest in elucidation of a functional unit that drives self-assembly of reflectin-based materials led us to develop an approach for systematic investigation of a library of reflectin-based protein sequences. Cassette-based DNA cloning and concatemerization techniques were used to synthesize genes encoding tandem repeats of reflectin-based amino acid sequences. Reflectin constructs of interest included a reflectin 1a domain 3 (D3) monomer, a domain 3 dimer, subdomain peptides, recombinant reflectin 1b, an elastin-reflectin diblock copolymer, and an elastin-reflectin-GFP fusion protein. After construction of the sequences of interest at the DNA level, protein expression was carried out in a bacterial host. Approximately 10-20 mg protein/L cell culture yields were obtained for each construct. Preliminary structural characterization was performed. The unique spectral properties associated with recombinant reflectin protein materials make elastin-reflectin chimeric protein polymers attractive potential targets for biomaterial engineering applications.

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Final Performance Report 2012

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Principal Investigator: Dr. Holly E. Carpenter Desai

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Subject: Final Performance Report to Dr. Hugh De Long

Contents

II. Abstract

Our interest in elucidation of a functional unit that drives self-assembly of reflectin-based materials led us to develop an approach for systematic investigation of a library of reflectin-based protein sequences. Cassette-based DNA cloning and concatemerization techniques were used to synthesize genes encoding tandem repeats of reflectin-based amino acid sequences. Reflectin constructs of interest included a reflectin 1a domain 3 (D3) monomer, a domain 3 dimer, subdomain peptides, recombinant reflectin 1b, an elastin-reflectin diblock copolymer, and an elastin-reflectin-GFP fusion protein. After construction of the sequences of interest at the DNA level, protein expression was carried out in a bacterial host. Approximately 10-20 mg protein/L cell culture yields were obtained for each construct. Preliminary structural characterization was performed. Collaborations established during the grant period provide valuable opportunities for continuing characterization work beyond the award period. Future experiments will include continued structural characterization of these novel protein-based biomaterials, construction of additional elastin-reflectin fusion chimeras, as well as construction of other block copolymer materials encoding reflectin sequences. The unique spectral properties associated with recombinant reflectin protein materials make elastin-reflectin chimeric protein polymers attractive potential targets for biomaterial engineering applications.

III. Objectives

i. Research Aim 1: Perform structural analysis of reflectin subdomain peptides, domain peptides, and full-length, reflectin-based proteins

ii. Research Aim 2: Achieve biosynthesis of variable length reflectin-based subdomain and domain multimers

- Choose suitable model proteins and perform characterization studies

iii. Research Aim 3: Construct diblock and triblock elastin-reflectin block copolymers

- Characterize reflectin-elastin protein polymers
- Evaluate the ability to purify polymers using the inverse temperature transition of elastin

IV. Findings/Results

Research Aim 1: Perform structural analysis of reflectin subdomain peptides, domain peptides, and full-length, reflectin-based proteins

Completed Goals:

A. In order to measure what role the repetitive, conserved subdomain sequence of reflectin proteins may have in self-assembly and structure of reflectin-based materials, structural characterization was performed on the recombinant reflectin 1a (subdomain 3, "SD3") peptide. The subdomain was hypothesized to serve as a functional unit for the structure of reflectins based on work by Crooks and coworkers[1]. Peptides were

synthesized via solid-phase peptide synthesis and were obtained from the Naik lab at the AFRL. The peptide was found to be soluble in a variety of aqueous solvents and buffer systems while full-length recombinant reflectin was insoluble in water. Circular dichroism was used to demonstrate that the peptide adopted random coil structure in a variety of aqueous environments (Fig. 1a) while the peptide displayed α-helical character when dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (Fig. 1b). These results support findings obtained in the Naik lab with another related peptide sequence. It is likely that the HFIP solvent induces α-helical secondary structure in the subdomain-based peptide and that the peptides are largely random coil.

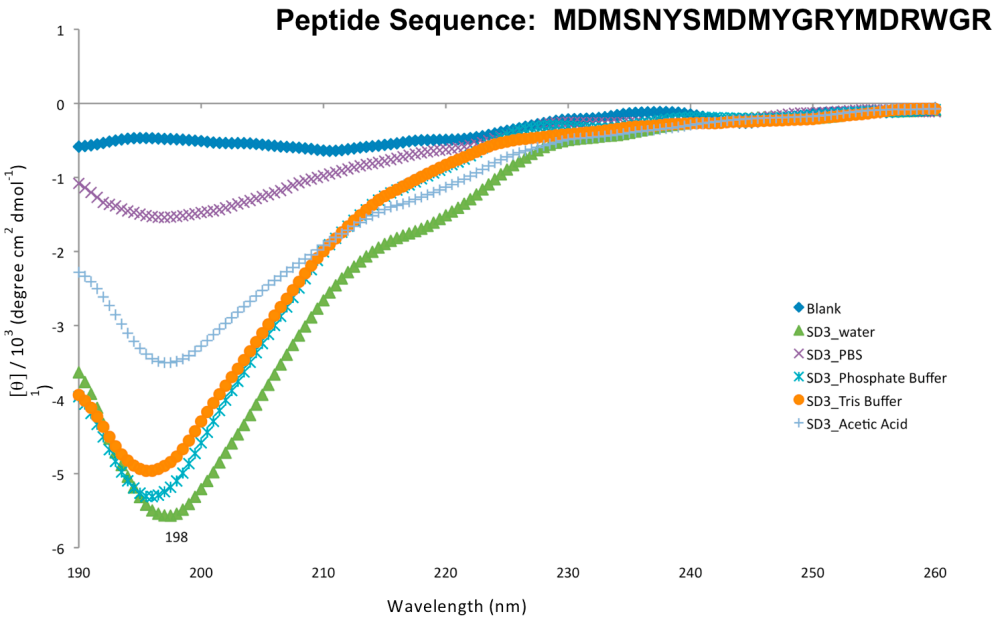


Figure 1.a. Circular dichroism analysis of the subdomain 3 (SD3) peptide. Peptides were dissolved in various solvents at a concentration of 2 mg/mL. Buffers were prepared at a concentration of 10 mM. Reflectin 1a subdomain peptides, synthesized via solid-phase peptide synthesis, exhibit random coil structure in a variety of solvent systems under variable conditions of ionic strength, pH, and peptide concentration.

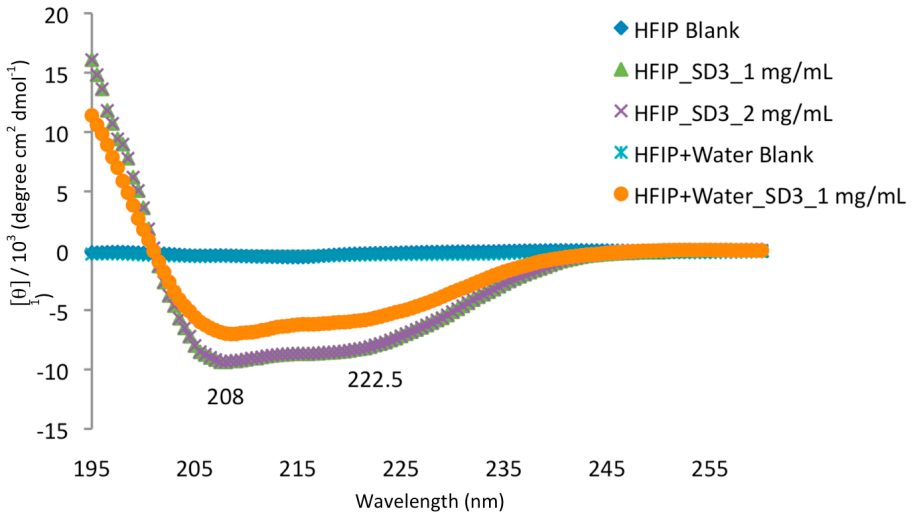
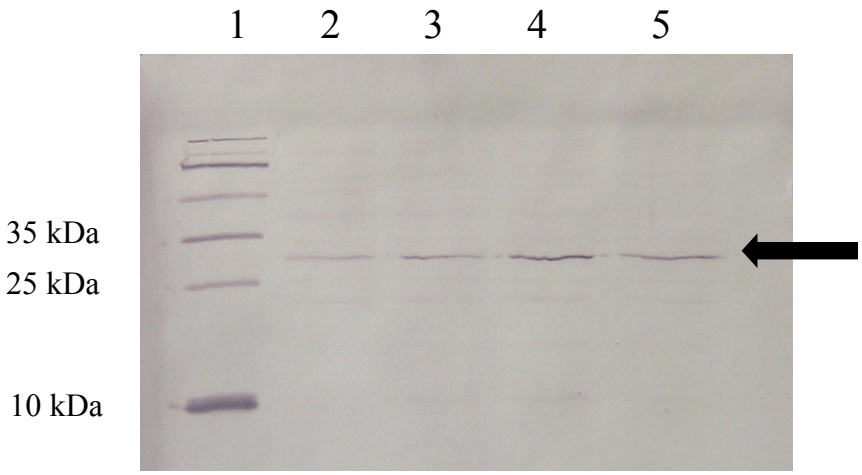


Figure 1.b. Circular dichroism analysis of the subdomain 3 (SD3) peptide. Peptides were dissolved in HFIP at concentrations of 1 or 2 mg/mL.

B. Expression of the full-length, recombinant reflectin 1b was performed in *E. coli* and purification was completed (Fig. 2).



1. Perfect protein marker
2. Before induction with IPTG
3. 1 h after IPTG induction
4. 2 h after induction
5. 3 h after induction

Figure 2. Western blot indicating expression of recombinant reflectin 1b protein. The protein was genetically engineered with a hexahistidine tag for purification. Expression levels of the protein appeared to be highest 2 h after induction.

C. In order to measure what role the repetitive, conserved domain sequences of reflectin proteins may have in self-assembly and structure of reflectin-based materials, recombinant DNA technology techniques were used to generate a gene encoding domain 3 (D3) of the reflectin 1a sequence. The domain was hypothesized to serve as a functional unit for the structure of reflectins based on work by Crooks-Goodson and coworkers[1]. As part of our collaboration with Dr. Crookes-Goodson and coworkers, we planned to focus on genetic engineering and protein expression of Domain 3 while the Dr. Crookes-Goodson team focused on investigating the properties of Domains 4 and 5. The D3 protein is 55 amino acids in length. Based on our observations reported on the random coil structure of subdomain peptides as determined by CD analysis, we decided to focus on biosynthesis and characterization of entire domain peptides. Rather than using solid-phase peptide synthesis, we generated a gene for expression of the D3 protein by annealing single-stranded DNA sequences and subsequent cloning into an expression plasmid (Fig. 3).

The expression plasmid was designed by cloning an adapter cassette (designed *de novo*) into a modified pQE60 bacterial expression plasmid. The adapter sequence (Fig. 4) was designed for the specific purpose of cloning and insertion of any reflectin-based monomer or polymer sequence that we generated due to the *Bsa* I restriction endonuclease sites within the cloning region of the adapter. The *Bsa* I enzyme has cut sites downstream of its recognition site and our unique method allows us to insert any reflectin or even elastin-mimetic gene sequence with the correct "TCCA" sticky ends at the 5′and 3′ ends of each gene. The adapter sequence encodes a decahistidine tag at the C-terminal end of any gene cloned into the adapter sequence. This adapter was also useful for cloning of the elastin-reflectin diblock sequence as described below under Aim 3. The D3 expression plasmid sequence was confirmed by automated DNA sequencing and the D3 monomer protein has been expressed in *E. coli* (Fig. 5). We are currently in the process of optimizing expression conditions and producing milligram to gram quantities of the purified protein for structural analysis. The protein (mg scale) has been purified by affinity chromatography using the 10x polyhistidine tag encoded at the C-terminus of the protein. In future studies, we plan to investigate the structure of the D3 protein by circular dichroism, Raman spectroscopy, and other techniques. For example, I invited Dr. Ray Tu at the City College of New York to come to North Georgia in January 2011 to give a seminar on his research. While visiting, he expressed an interest in investigating the mechanical properties of the D3 domain protein as well as other reflectin-based proteins we are generating as they compare to the full-length recombinant reflectin protein.

D. Expression of the full-length, recombinant reflectin 1b is currently underway and we are expressing this full-length protein at high yield in order to compile milligram to gram quantities of the protein for structural analyses. The properties of purified reflectin 1b will be investigated as compared to results from the Domain 3 monomer protein as well as from our D3 dimer construct (See Aim 2 below).

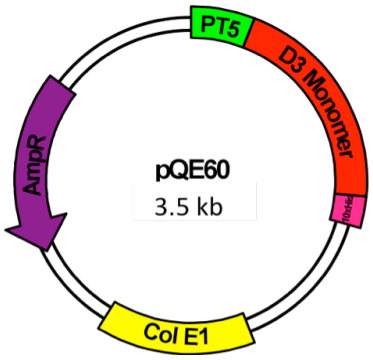


Figure 3. Reflectin Domain 3 (D3) expression plasmid sequence

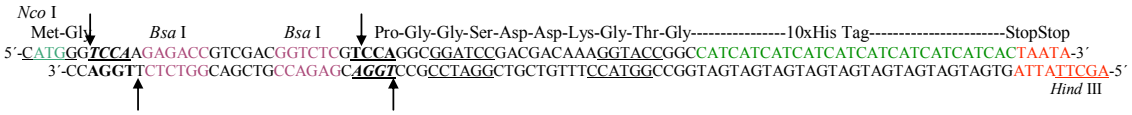


Figure 4. Versatile adapter sequence used for cloning reflectin-based and elastin-mimetic gene sequences into the pQE60 expression plasmid. The adapter was generated by annealing single-stranded DNA sequences corresponding to the desired nucleotide sequence and the entire construct was cloned into the pQE60 plasmid using the *Nco* I and *Hind* III restriction endonuclease sites. The adapter allows for cloning of DNA sequences in the final expression plasmid and incorporation of a C-terminal decahistidine tag for purification. This adapter was also recently used for cloning a diblock elastin-reflectin fusion polymer (See Aim 3 below).

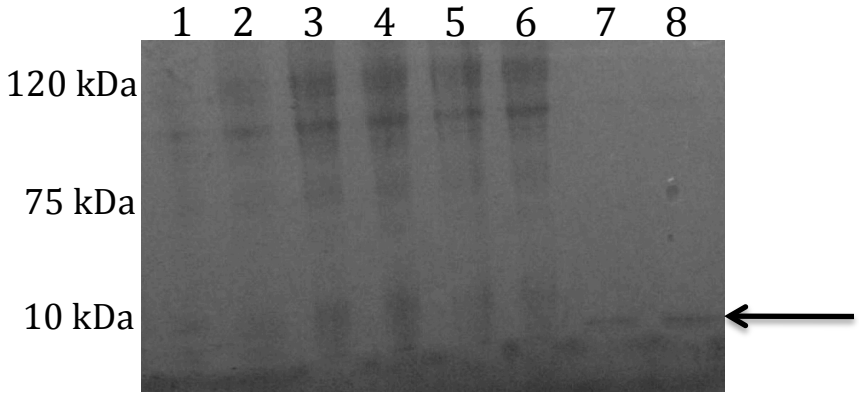


Figure 5. SDS-PAGE gel indicating expression and purification of recombinant reflectin 1a Domain 3 (D3) protein. The protein was tagged with a decahistidine tag for purification. The purified D3 protein (MW~7 kDa) is present in lanes 7 and 8 (arrow). Lane 1: Expression level before IPTG induction; Lane 2: Cell lysate 1 hr after induction;

Lanes 3 and 4: Cell lysate 2 hr after induction; Lanes 5 and 6: Cell lysate 3 hr after induction; Lanes 7 and 8: Purified D3 protein monomer (Yield ~30 mg/L cell culture)

Research Aim 2: Achieve biosynthesis of variable length reflectin-based subdomain and domain multimers

-Choose suitable model proteins and perform characterization studies

Completed Goals:

A. Several reflectin-based genes were designed and constructed at the DNA level in an attempt to understand more about the primary sequence dependence of reflectin domain sequences in the structure and self-assembly of reflectin-based materials. Expression and preliminary characterization of these model reflectin-based constructs followed the initial synthesis/construction phase. Reflectin-based genes and purified protein will be shared with and further characterized by Dr. Rajesh Naik's group and Dr. Wendy Crookes-Goodson's team at the Air Force Research Lab as part of a collaborative effort aimed at elucidating the structure and potential applications of reflectin-based biomaterials.

B. Repetitive polymers (multimers) of the reflectin 1a subdomain 2 (SD2) sequence were generated at the DNA level using a DNA concatemerization method (Fig. 6 and Fig. 7) that would allow for seamless cloning of polymers of variable length based on the SD2 monomer sequence. Several multimeric DNA polymers encoding the SD2 repeat sequence have been isolated via extraction from an agarose gel (Fig. 8). Difficulty cloning these sequences into an expression plasmid was encountered possibly due to the repetitive nature of these DNA sequences and the increased likelihood of secondary structural features inhibiting efficient cloning of longer repeat sequences. One goal of this ongoing work is to obtain longer repetitive polymers (5-10 repeats) of the subdomain sequences.

C. Two novel reflectin-based genes were designed and constructed at the DNA level using a seamless cloning strategy (Fig. 6). Dimeric repeat sequences of subdomain 2 (SD2) and domain 3 (D3) were obtained and the D3 dimer gene was cloned into an expression plasmid (Fig. 7). The D3 dimer gene consists of two tandem repeats of the domain 3 sequence of reflectin 1a (~110 amino acids in length). This dimeric sequence does not have any linker region (additional amino acids) between the two D3 monomers due to our seamless cloning strategy. Although full-length recombinant reflectin consists of 5 repetitive domains, the protein is highly insoluble in water and gives rise to difficulty in structural investigations of this material, similar to challenges encountered in early studies on the structural protein elastin. It was hypothesized that shorter repeat polymers would exhibit improved water solubility. Following expression and purification, our D3 dimeric protein was found to be insoluble in water which was consistent with similar evidence from other dimeric sequences (Domain 3, 4 and 5 dimers) made by the Crookes-Goodson group (personal communication). Protein expression of the D3 dimer construct was achieved and purification typically yielded 10-20 mg protein/L of cell culture. Reflectin-based genes and purified protein will be shared with and further characterized by Dr. Rajesh Naik's group at the Air Force Research Lab as part of a collaborative effort aimed at elucidating the structure and potential applications of reflectin-based biomaterials.

i. A dimeric gene of the reflectin 1a subdomain 2 (SD2) sequence was generated at the DNA level using a DNA concatemerization method that allowed for seamless cloning of polymers of variable length based on the SD2 monomer sequence. The SD2 gene is currently being cloned into an expression plasmid as part of ongoing work.

ii. A reflectin 1a Domain 3 (D3) sequence dimer was generated at the DNA level using a DNA concatemerization method that allowed for seamless cloning of polymers of variable length based on the D3 monomer sequence. The D3 dimer was cloned into an expression plasmid (Fig. 7.a) and protein expression and purification were achieved.

D. Although larger polymers of the D3 and SD2 sequences were generated at the ligation step and were visible via agarose gel electrophoresis (Fig. 8), we had difficulty cloning the larger SD2 and D3 (tetramers, pentamers, hexamers, and larger) constructs into expression plasmids. Sequence repetitive DNA monomers encoding longer structural protein sequences are often difficult to clone due to sequence instability in the *E. coli* host even in recombination deficient strains. As a solution, we plan to use a stepwise, recursive directional ligation strategy in order to obtain larger D3 constructs such as tetramer and octamer sequences (Fig. 10). The D3 dimer we have already constructed will likely serve as a model protein that gives insight into amino acid sequence dependence of reflectin-based proteins. Repeating a single domain sequence 2-10 times, compared to the 5 different domains in the native reflectin, will likely aid in understanding more about the functional unit and primary sequence dependence for reflectin structure and self-assembly.

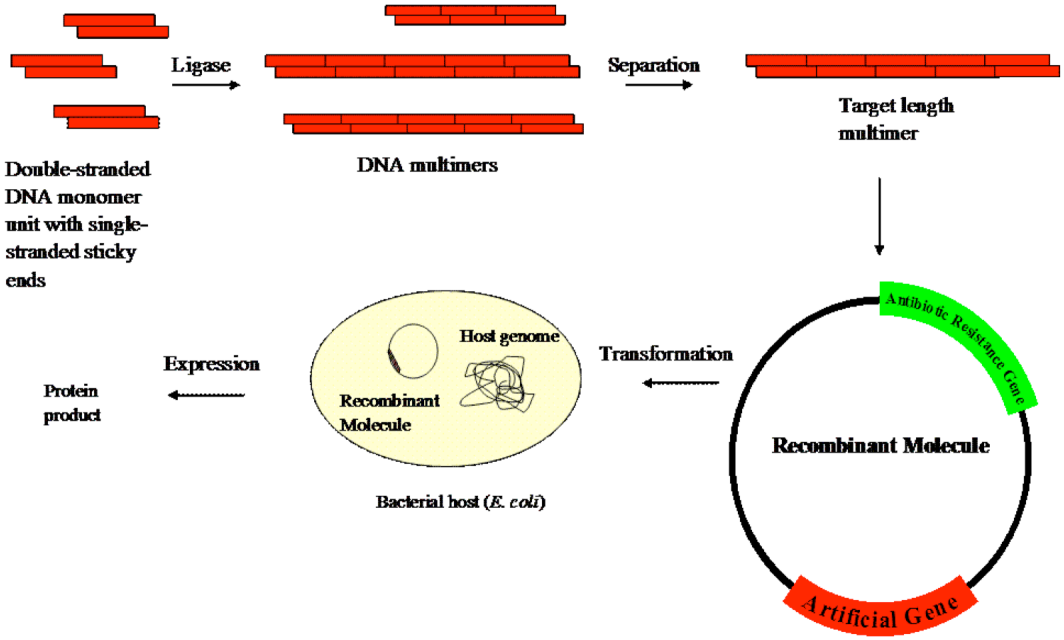


Figure 6. Outline for the DNA cassette concatemerization method used to generate reflectin-based multimers. DNA monomer sequences are synthesized and annealed to generate double-stranded DNA fragments with complementary sticky ends. A DNA ligase enzyme is used to covalently link monomers together to form polymers of different

lengths. DNA polymers are separated using gel electrophoresis and multimers of the desired length are cloned into an expression plasmid. Multimer protein expression is achieved in a bacterial host.

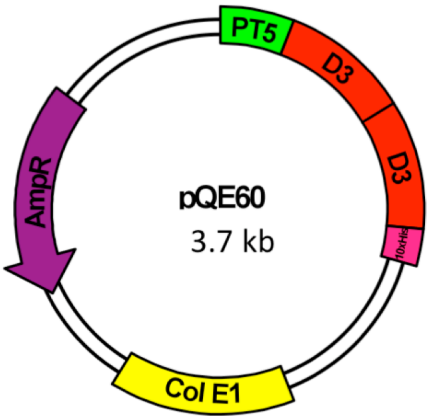


Figure 7. a. Expression plasmid containing the reflectin 1a domain 3 (D3) dimer gene construct.

Reflectin 1a Domain 3 Monomer-Forward Sequence
5′-AGCTTGAAGACAATCCATATAGCCAGTGGATGATGTATAACTATAACCGCCATGGTTATTA
CCCGAACTATAGCTACGGCCGCCATATGTTTTATCCGGAACGCTGGATGGATATGAGCAACTA
TAGCATGGATATGTATGGCCGTTATATGGATCGCTGGGGCCGCTATTGCAATCCAAGAGA
CGG-3′

Reflectin 1a Domain 3 Monomer-Reverse Sequence
5′-GATCCCGTCTCTTGGATTGCAATAGCGGCCCCAGCGATCCATATAACGGCCATACATATCC
ATGCTATAGTTGCTCATATCCATCCAGCGTTCCGGATAAAACATATGGCGGCCGTAGCTATAG
TTCGGGTAATAACCATGGCGGTTATAGTTATACATCATCCACTGGCTATATGGATTGTCT
TCA-3′

Figure 7. b. DNA sequences corresponding to the reflectin 1a domain 3 monomer sequence. Single-stranded DNA, encoding domain 3 monomer amino acid sequences are shown in red. Complimentary DNA sticky ends, shown underlined, are covalently ligated to generate polymers corresponding to D3 multimer proteins according to the strategies shown in Figures 6 and 10.

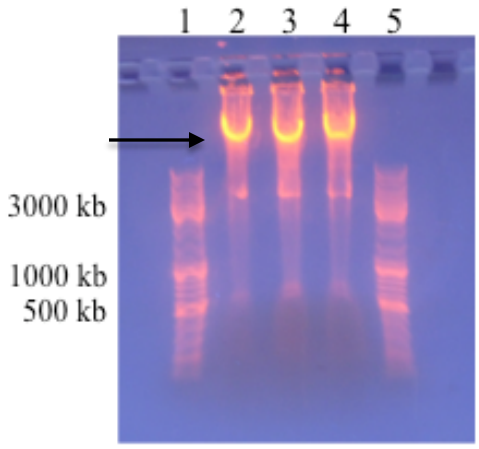


Figure 8. 1 % agarose gel indicating the results of multimerization of reflectin 1a D3 monomer DNA sequences after the ligation step. Reflectin D3 DNA multimers appear as ladders in lanes 2, 3, and 4. Each ladder represents DNA polymers of different lengths based on the D3 monomeric repeat. DNA size markers (Lanes 1 and 5) flank multimer lanes to serve as reference points for the size of resultant multimer sequences. The resultant multimers of interest between 3000 and 750 bp in length correspond to between 15 and 5 repeat monomers. A significant population of larger constructs was observed (arrow) but difficulty in cloning larger constructs was encountered possibly due to sequence instability in *E. coli*.

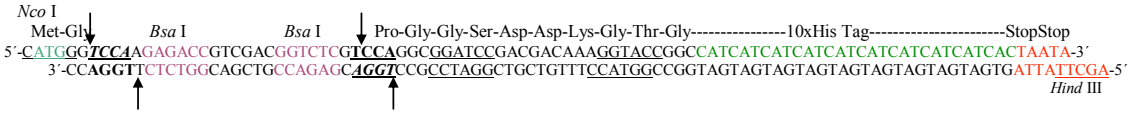


Figure 9. DNA sequence of the adapter that has been designed and annealed for cloning of the D3 multimers obtained. The adapter is similar to the adapter that was designed for the SD2 multimers and allows for cloning of DNA sequences in the final expression plasmid and incorporation of a C-terminal decahistidine tag for purification. This adapter is also being used for cloning diblock and triblock elastin-reflectin polymers.

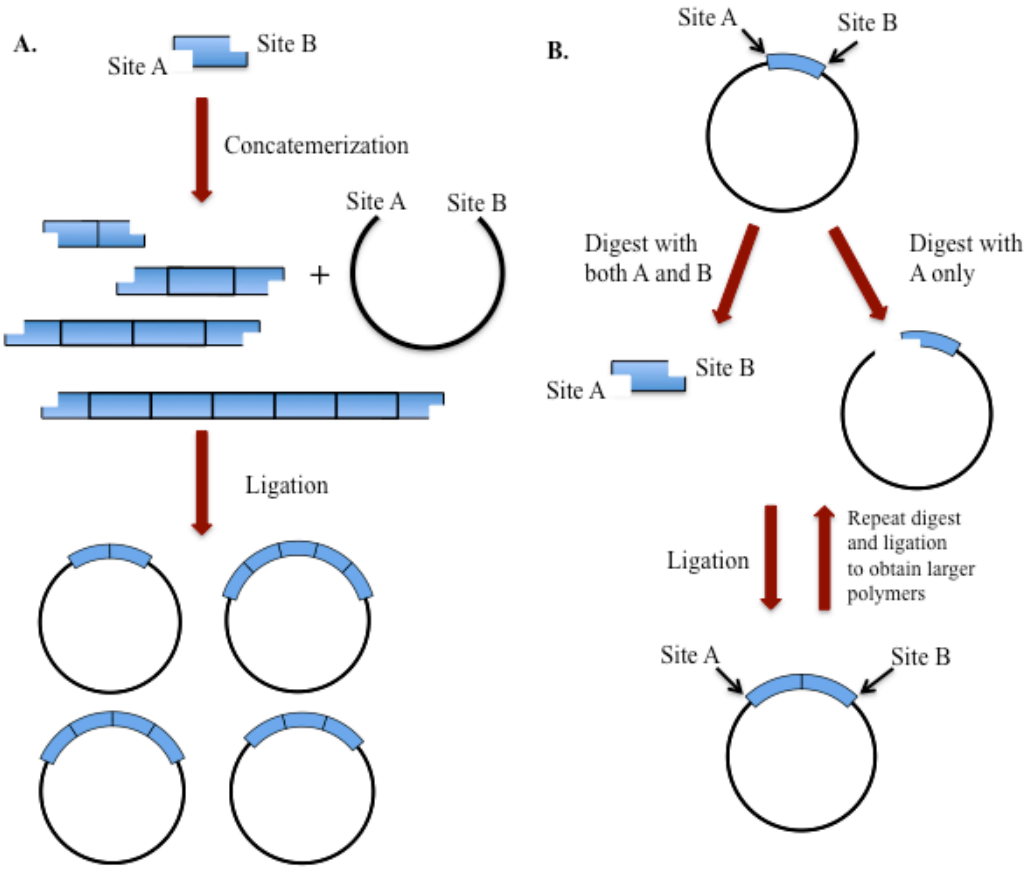


Figure 10. Strategies for synthesis of polymeric proteins at the DNA level. DNA cassette concatemerization (**A**) and recursive directional ligation[2] (**B**) are methods used for synthesis of polymeric proteins. The blue blocks represent DNA monomers that encode D3 monomeric repeats. Sites A and B represent locations where restriction endonucleases cut the DNA to afford complimentary sticky ends used for multimerization (polymerization) or for cloning into the expression plasmid.

Research Aim 3: Construct diblock and triblock elastin-reflectin block copolymers

- Characterize reflectin-elastin protein polymers
- Evaluate the ability to purify polymers using the inverse temperature transition of elastin

Completed Goals:

A. A diblock elastin-reflectin copolymer gene has been designed at the DNA level and cloned into an expression plasmid (Fig. 11). Expression and purification of the elastin-reflectin hybrid construct has begun (Fig. 12). As part of this ongoing effort, we are currently optimizing expression conditions in order to maximize the yield of the elastin-reflectin diblock protein. It was hypothesized that elastin-mimetic protein fusions to reflectin proteins would enable increased water solubility of the materials and enable subsequent purification of the chimeric proteins via utilization of the inverse temperature transition characteristic of elastin-mimetic materials. The diblock itself displays limited water solubility and low bacterial expression yields likely due to the overall size (~100 kDa) and largely hydrophobic nature of the polymer. Although we are currently purifying the elastin-reflectin diblock protein via affinity chromatography and a C-terminal decahistidine tag on the protein under denaturing conditions, we plan to isolate the protein via the temperature cycling procedure (outlined in Fig. 13) used for purification of elastin-like proteins once expression conditions have been optimized.

Diblock elastin-like protein constructs of alternating hydrophobic and hydrophilic blocks are known to self-assemble into micelles or nanoparticles based on the lower critical solution behavior of elastin-mimetic proteins (Fig. 14). Triblock elastin-mimetic proteins are known to self-assemble to form hydrogel materials. In addition, triblock constructs may be chemically cross-linked to further increase the mechanical strength of the hydrogels produced. Block copolymers with sequences of variable plastic and elastic domains, as in the case of the diblock we have designed, will likely provide for a new class of reflectin-based materials. Reflectin-based genes and purified protein will be shared with and further characterized by Dr. Rajesh Naik's group at the Air Force Research Lab as part of a collaborative effort aimed at elucidating the structure and potential applications of reflectin-based biomaterials.

i. Three different elastin-mimetic DNA sequences were obtained from Dr. Vince Conticello's group at Emory University for use in construction of elastin-reflectin-based materials. Each elastin-mimetic sequence of significant length has variable material properties based on the unique sequence of each construct (Fig. 15).

ii. A diblock cloning strategy was developed for making a novel elastin-reflectin protein fusion, and the method allowed for cloning of full-length reflectin 1b adjacent to a hydrophilic elastin-mimetic polymer at the DNA and protein levels. Additional reflectin-based dimers or multimers (D3 or SD2) can be cloned adjacent to the elastin gene as well. The existing diblock construct fuses the full-length reflectin 1b gene adjacent and downstream of a hydrophilic elastin-mimetic polymer at the DNA and

protein levels. The amphiphilic block copolymer that results will be characterized following optimization of expression conditions.

iii. The adapter designed for cloning of the D3 multimer genes (Fig. 9) was used for cloning the diblock polymer that consists of an elastin-mimetic sequence fused to full-length reflectin 1b.

iv. A triblock cloning strategy that is very similar to diblock cloning (Fig. 16) has been developed with utilization of the designed complementary sticky ends of elastin and reflectin-based sequences. It is hypothesized that the triblock sequence will result in a protein polymer that can be used to generate a reflectin-containing hydrogel material with potentially useful spectral properties. Cloning of this triblock is currently underway as part of this ongoing research effort.

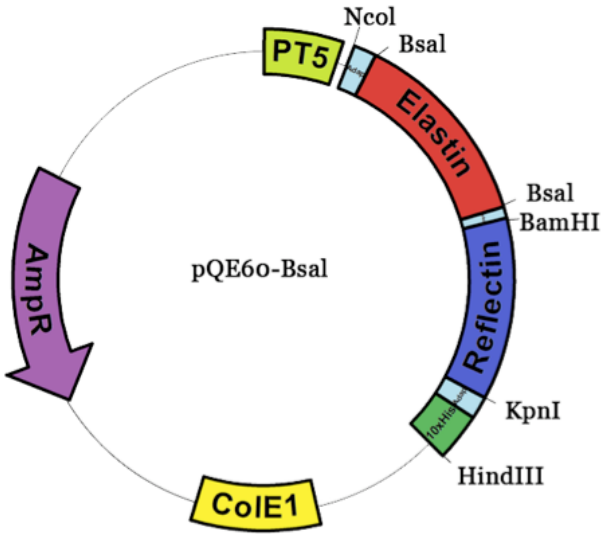


Figure 11. Expression plasmid containing the diblock elastin-reflectin fusion amphiphilic block copolymer gene construct. Diblock elastin-like protein constructs of alternating hydrophobic and hydrophilic blocks are known to self-assemble into micelles or nanoparticles based on the lower critical solution behavior of elastin-mimetic proteins (Fig. 14). It is hypothesized that the diblock and/or a triblock sequence will result in a protein polymer that can be used to generate an elastin-reflectin-containing hydrogel material with potentially useful spectral properties.

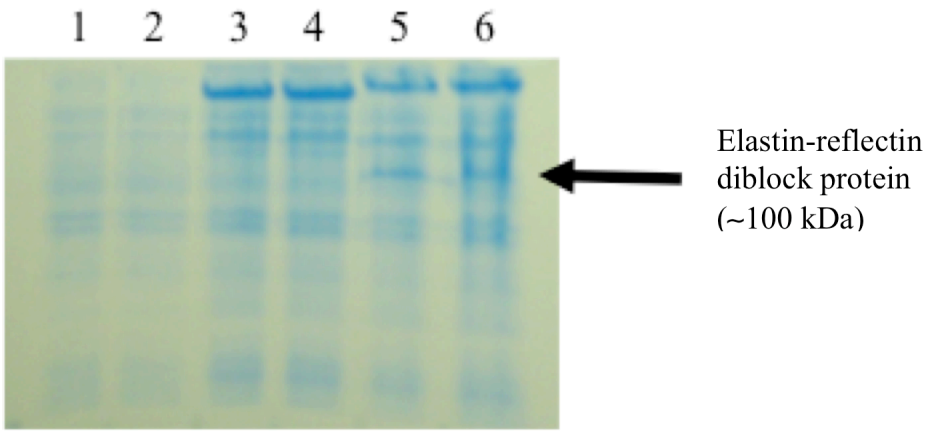


Figure 12. SDS-PAGE gel indicating expression of the elastin-reflectin fusion diblock protein. The protein was tagged at the C-terminus with a decahistidine tag for purification. Lanes 1 and 2: Expression level before IPTG induction; Lanes 3 and 4: Cell lysate 1 hr after induction; Lanes 5 and 6: Cell lysate 2 hr after induction

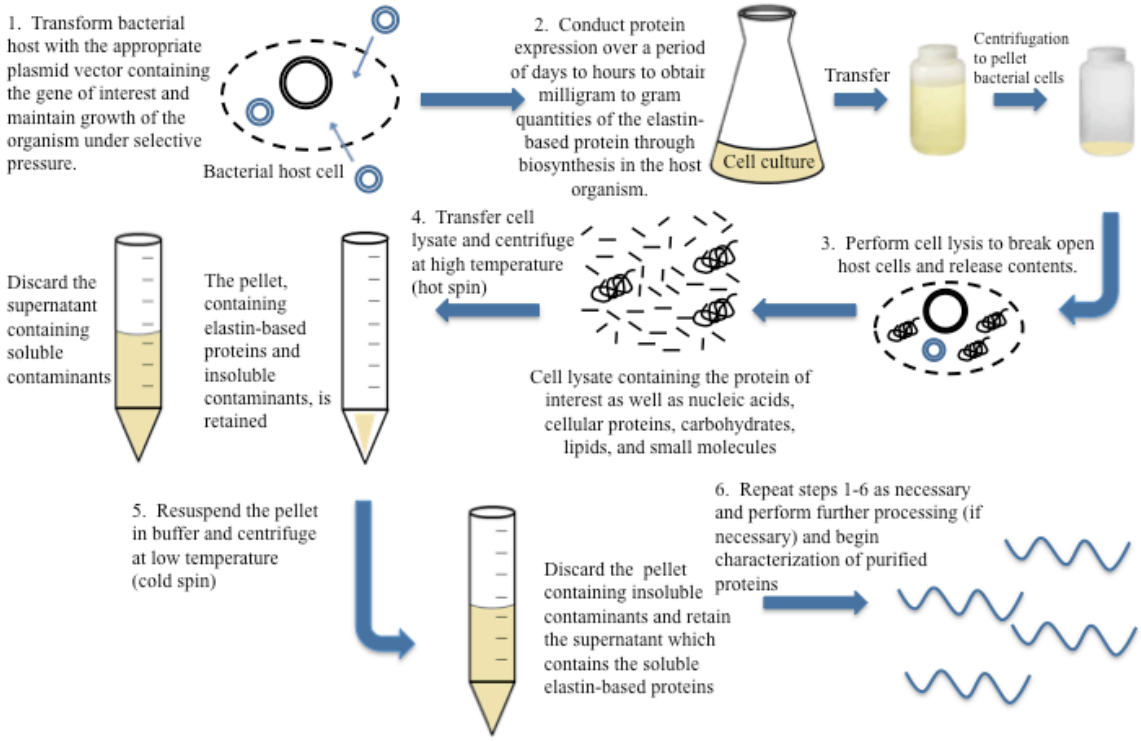


Figure 13. Biosynthesis and purification of elastin-like proteins (ELP) from a bacterial host by inverse temperature transition cycling[3]. The inverse temperature phase transition of the ELP is induced by raising the temperature of the cell lysate during the first hot spin (37-42 °C). The aggregated ELP is retained in the pellet, resolubilized in cold buffer, and centrifuged at a low temperature (4 °C). After the cold spin, the insoluble contaminants are removed and the soluble ELP is left in the supernatant. Repetitive cycles (steps 1-6) of this procedure can further increase the purity of the ELP or ELP-fusion protein solution.

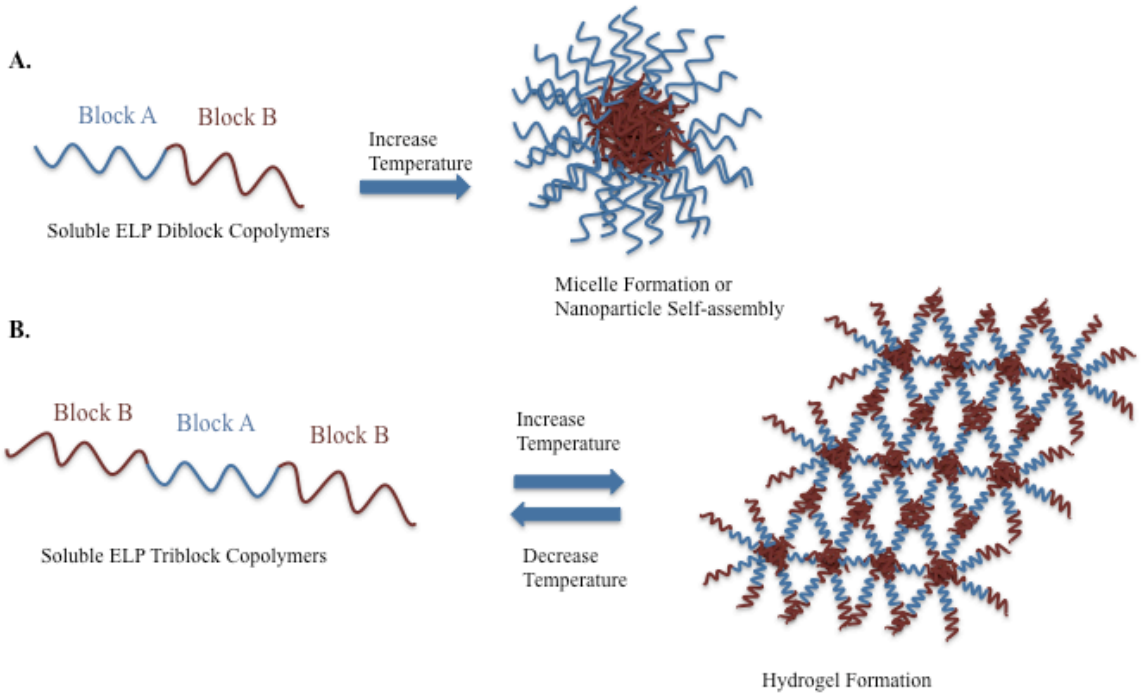


Figure 14. Thermal response of diblock and triblock elastin-like protein (ELP) copolymer constructs. **A.** Diblock copolymers composed of hydrophilic ELP sequences (Block A, blue) and hydrophobic ELP polymeric sequences (Block B, red) form micelles in aqueous solution above the T_t of the Low T_t, elastic or hydrophobic block B. The hydrophilic, plastic block A remains in solution as the temperature of the solution remains below the characteristic T_t for block A. In **B.** below, triblock copolymers composed of alternating (BAB or ABA) sequences are capable of hydrogel formation through reversible self-assembly and hydrophobic collapse of endblock domains. Virtual crosslink formation is driven by micro-phase separation of identical endblock domains above their T_t[4].

- **Hydrophilic Elastin Block: $[(VPGAG)_4VPGEG]_{20}$**
- **Hydrophobic Elastin Block: $(IPAVG)_{20}$**
- **Cross-linkable Elastin Block: $[(VPGVG)_4VPGYG]_{20}$**

Figure 15. Amino acid sequences of three different elastin-mimetic proteins that have been obtained for construction of elastin-reflectin based fusion proteins. Each elastin-mimetic sequence of significant length has variable material properties based on the unique sequence of each construct.

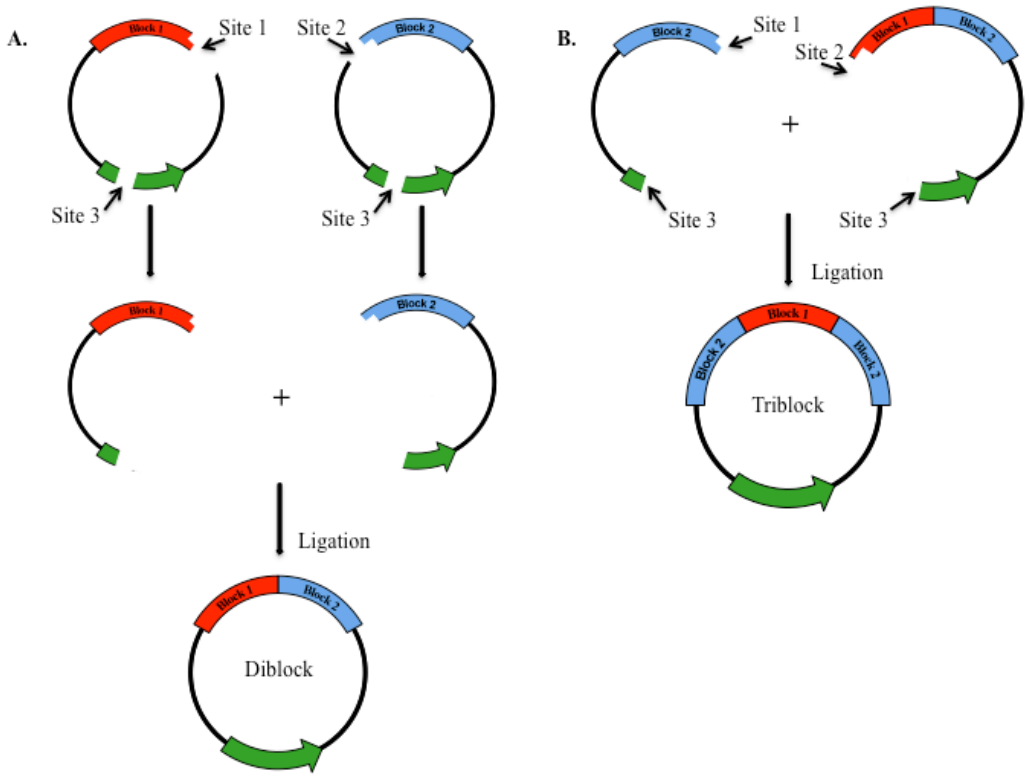


Figure 16. Cloning strategy devised for fusion of reflectin-based multimers to elastin-mimetic sequences. Block 1 represents a hydrophilic elastin domain while Block 2 represents a hydrophobic reflectin domain or vice versa. Sites 1, 2, and 3 represent sites at which restriction endonuclease enzymes cleave the DNA to produce specific, complimentary sticky ends for ligation of target DNA segments. Site 3 cuts specifically in the antibiotic resistance gene to afford increased selective pressure for the desired ligation product. The method allows for diblock or triblock cloning of reflectin adjacent to an elastin at the DNA and protein levels.

V. Supported Personnel

Principle Investigator: Holly E. Carpenter Desai, 9 week Summer Salary 2009, 2010, 2011

Students: This grant funded stipends for 6 undergraduate students in Summer (9 week) Research Assistantship Positions

Whitney Holcombe 2009
Noelle Conneely 2009
Rebecca Bartlett 2010
Brian Young 2010
Rebecca Bartlett 2011
Patrick Pickens 2011

VI. Collaborations

1. Collaboration with Vince Conticello, Ph.D., Emory University

A collaboration was established by the PI with Vince Conticello at Emory University for use of instrumentation (CD) and for exchange of materials and elastin-mimetic gene sequences.

2. Collaboration with Rajesh Naik, Ph.D.* and Wendy Crookes-Goodson, Ph.D.*, AFRL

A collaboration was established by the PI with Rajesh Naik and Wendy Crookes-Goodson at the Air Force Research Lab for exchange of materials including plasmids and reflectin-based gene sequences, reflectin-based proteins, and helpful discussions.

3. Collaboration with David Kaplan*, Ph.D., Tufts University

A collaboration was established by the PI with David Kaplan at Tufts University for exchange of materials including plasmids and reflectin-based gene sequences, reflectin-based proteins, and helpful discussions. An undergraduate student from North Georgia was employed in the Kaplan lab in a paid summer internship June 1, 2010—August 1, 2010 at Tufts University. Dr. Kaplan was invited to give a research seminar at North Georgia in fall 2010.

4. Collaboration with Dimitri Deheyn*, Ph.D., Scripps Institution of Oceanography

A collaboration was established by the PI with Dimitri Deheyn at Scripps Institution of Oceanography for the use of his hyperspectral imaging instrumentation in the characterization of reflectin-based proteins and for helpful discussions. The PI and an undergraduate student from North Georgia have secured award funding from a North Georgia Travel Award and a Burroughs Wellcome Foundation Collaborative Travel Grant for the summer of 2012 to travel to Scripps. From May 5 to June 30, 2012, the PI will be working in collaboration with Dr. Deheyn to further characterize reflectin-based materials as well as to facilitate biochemical characterization and protein expression of protein materials of interest from the *Chaetopterus* marine worm. Dr. Deheyn was invited to give a research seminar at North Georgia in spring 2011.

5. Collaboration with Kenneth Sandhage*, Ph.D., Georgia Institute of Technology

A collaboration was established by the PI with Ken Sandhage at GA Tech in an effort to coat the surface of diatom frustules with reflectin-based protein materials. The Sandhage lab has provided our group with helpful discussions and several samples of cleaned diatom frustules from 4 different species for our preliminary coating experiments. This collaboration will extend beyond the end of this AFOSR grant period. Dr. Sandhage was invited to give a seminar at North Georgia in January 2012.

6. Collaboration with Mark Hildebrand*, Ph.D., Scripps Institution of Oceanography

After a collaboration was established with Dr. Dimitri Deheyn, the PI traveled to Scripps in July 2011 to meet with the members of the Deheyn lab. While there, Dr. Deheyn suggested a meeting between the PI and Dr. Hildebrand in an effort to express reflectin in diatoms. Based on this idea from Dr. Deheyn and discussion with

Wendy Crookes-Goodson, a collaboration was established with the Hildebrand lab for exchange of a reflectin gene for cloning and expression of full-length reflectin 1b in diatoms. This research is ongoing beyond the end of this grant period and the PI will travel to Scripps May-June, 2012 to continue this collaboration.

*Indicates other AFOSR Funded Individuals

VII. Publications

Invited Comprehensive Review Article:
Conticello, V. P. and **Carpenter Desai, H. E.** "Elastins," *Comprehensive Polymer Science*, 2nd ed., Vol. 9 (2012), Accepted for publication. (Please see proof included at the end of this report.)

VIII. Professional Presentations

Invited Presentations

Carpenter Desai, H. E. Biosynthesis and characterization of novel reflectin-based protein polymers," Oral Presentation. 243rd *National Meeting of the American Chemical Society*. San Diego, CA, March 25, 2012.

Carpenter Desai, H. E. Biomaterials Engineering: Reflectin-based protein polymers. Oral Presentation. *Scripps Institution of Oceanography*, Marine Biology Research Division. La Jolla, CA, June 29, 2011.

Carpenter Desai, H. E. Novel Reflectin-Mimetic Protein Materials. Oral Presentation. *Francis Marion University*, Department of Chemistry. Florence, SC, April 8, 2010.

Carpenter, H. E. Development of Novel Reflectin-Mimetic Protein Materials. Oral Presentation. *Tufts University*, Department of Biomedical Engineering. Medford, MA, May 7, 2009.

Presentations at Professional Meetings

Carpenter Desai, H. E., Bartlett, R., Young, B., and Pickens, P. Reflectin-based protein polymers: Biosynthesis and characterization," Poster. 243rd *National Meeting of the American Chemical Society*. San Diego, CA, March 27, 2012.

Carpenter Desai, H. E., Bartlett, R., Young, B., and Pickens, P. "Biomaterials engineering: Synthesis and characterization of novel reflectin-based protein polymers," Poster. 242nd *National Meeting of the American Chemical Society*. Denver, CO, August 28-31, 2011.

Carpenter Desai, H. E., Bartlett, R., Pickens, P., Young, B., Jones, M., and Husk, Z. "Design and Biosynthesis of Novel Reflectin-Based Biomaterials," Poster. 43rd *IUPAC World Chemistry Congress*. San Juan, Puerto Rico, July 30-August 5, 2011.

Carpenter Desai, H. E. , Husk, Z., Young, B., and Herbert, B. "Development of a problem-based case study for HPLC and GC/MS comparative analysis of caffeine levels in energy drinks," Poster. 62nd *Southeast Regional Meeting of the American Chemical Society*. New Orleans, LA, November 30-December 4, 2010.

Carpenter Desai, H. E., Young, B., Bartlett, R. and Peña, G. "Biomaterials Design: Reflectin-mimetic protein polymers," Poster. 240th *National Meeting of the American Chemical Society*. Boston, MA, August 22-26, 2010.

Carpenter Desai, H. E., Holcombe, W., and Conneely, N. "Design and biosynthesis of novel reflectin-mimetic protein materials," Poster. 239th *National Meeting of the American Chemical Society*. San Francisco, CA, March 21-24, 2010.

Carpenter Desai, H. E., Holcombe, W., and Conneely, N. "Biosynthesis of Novel Reflectin-Mimetic Protein Materials," Poster. 61st *Southeast Regional Meeting of the American Chemical Society*. San Juan, PR, October 21-24, 2009.

IX. Interactions/Transitions/Other Impacts

Educational Impacts and Awards: Funding from this AFOSR grant enabled training of 6-9 undergraduate students in scientific research. Two students worked full-time in the summers of 2009, 2010, and 2011 and an additional three worked part-time throughout the year on the project. In addition to the students paid by this grant in 2011, two more were paid in a 6 week summer internship funded by North Georgia in an institutional grant awarded in January 2011 from North Georgia's Center for Undergraduate Research (CURCA) in support of this project. All of these students continued their work in the research lab beyond the paid summer internships funded by the AFOSR grant. In addition, several awards have been made to our group by North Georgia for purchase of required instrumentation and additional supplies. This research has resulted in travel by the PI and by students to attend professional scientific meetings at the local, regional, national, and international levels to present and promote the AFOSR funded research. The PI applied for and was awarded an IUPAC Young Scientist Travel Award to attend and present the results of this research at the 43rd *IUPAC World Chemistry Congress* in San Juan, Puerto Rico, July 30-August 5, 2011. Over 16 separate oral or poster presentations were given by students and 7 presentations were given by the PI on this research.

Continuation of funding beyond the grant period: Departmental funding from the Department of Chemistry at North Georgia College & State University has been provided to continue funding this research during the spring and fall semesters of 2012-2013. The PI and an undergraduate student from North Georgia have secured award funding from two separate North Georgia Travel Awards and a Burroughs Wellcome Foundation Collaborative Travel Grant (total funding in the amount of $20,000) for the summer of 2012 to travel to Scripps. From May 5 to June 30, 2012, the PI will be working in collaboration with Dr. Deheyn to further characterize reflectin-based materials as well as

to facilitate biochemical characterization and protein expression of protein materials of interest from the *Chaetopterus* marine worm. The PI and student will also be working with the Hildebrand lab, while at Scripps, to facilitate cloning, expression, and characterization of full-length reflectin 1b in diatoms.

X. References

1. a. Crookes, W. J.; Ding, L. L.; Huang, Q. L.; Kimbell, J. R.; Horwitz, J.; McFall-Ngai, M. J., *Science,* **2004,** *303,* (5655), 235. b. Kramer, R. M.; Crookes-Goodson, W. J.; Naik, R. R. *Nature Materials,* **2007**, *6*, 533-538.
2. McDaniel, J. R.; Mackay, J. A.; Quiroz, F. G.; Chilkoti, A., *Biomacromolecules,* **2010,** *11,* (4), 944.
3. (a) Furgeson, D. Y.; Dreher, M. R.; Chilkoti, A., *J. Control Release,* **2006,** *110,* (2), 362; (b) McPherson, D. T.; Xu, J.; Urry, D. W., *Protein Expr. Purif.,* **1996,** *7,* (1), 51.
4. Wright, E. R.; Conticello, V. P., *Adv. Drug Deliv. Rev.,* **2002,** *54,* (8), 1057.